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NUCLEAR STRUCTURE AND SPORE FORMATION IN MICROSPHAERA ALNI

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Nuclear Structure and Spore formation
in Microsphaera Alni.

The literature on the development of the ascus and its ~~its~~ cytology has been recently and fully reviewed (4,13), and I shall only refer to such very recent papers as touch upon the points with which I have been specially concerned.

Faull (5) describes for Hydnobolites, Neotiella, Sordaria and some other species, a method of spore formation which he considers essentially different from that described by Harper (9,11).

He finds the primary nucleus always surrounded by the so-called metachromatic bodies, which Faull believes are normal cell products since they disappear in later stages. It is to be noted, however, that this disappearance may merely indicate that fixation is more perfect at these later stages, and I have so interpreted the appearance of similar bodies at certain stages in Microsphaera.

In Sordaria certain of the resting nuclei show centers, but in the other species centers with asters appear only at the time of division, disappearing in resting stages. Faull believes that the spindles are strictly intra nuclear in origin, as the poles are centers from which the long

astral rays extend. Each successive mitosis shows greater lengthening of the central spindle, till the nuclei resulting from the third division are brought very near the ascus wall. In the last division Faull finds the same persistence and subsequent bending of the astral rays as has often been described for spore formation, but attaches no importance to the rays or their activity as far as spore formation is concerned.

The first indication of spore formation, according to Faull, is the appearance of a specialized layer of cytoplasm beginning just around the center and developing progressively outward and around the nucleus until it encloses the cytoplasm of the future spore. He compares this limiting layer to the hyaline zones formed in the cleavage stages of the proto spores of Pilobolus (12).

It thins out from the center, stains differently from the cytoplasm, but is never clearly definable -" a hyaline zone, structureless or very finely granular." Two plasma membranes develop simultaneously on the site of the limiting layer, one about the spore-plasm, the other lining the cavity in which the spore lies. This produces no visible change in the limiting layer, although he thinks it probable that the plasma membranes result from a cleavage of the zone.

The nucleus "grows down into the center of the spore", forming a beak. Just what Faull means here by "growth" is very uncertain, especially as there is no mention of an increase in the size of the nucleus. Harper (11) has suggested several possible methods of beak formation, and seems to regard the activity of the astral rays, in bending down and exerting a pressure on the nucleus, as the most plausible.

When the exospore is formed, the nucleus resumes its spherical shape by withdrawing its beak and with it the center and aster, and in this Faull sees conclusive evidence that the rays take no part in the formation of the spore membrane.

Faull's figures of the beaked nucleus with its center and aster all within the spore membrane are very much like the polar or part polar views of spore formation which I have seen in Microsphaera where spore delimitation is certainly accomplished by means of the astral rays. I shall discuss the significance of such polar views further on. Faull however regards these figures as proof that the spores are not delimited by the fusion of kinoplasmic fibers, and leaves the question without accounting in any way for the persistence and bending of the rays during the process of spore formation.

Maire's latest paper (19) describes the nuclear division in a number of asci, the mitoses in all of which vary only in minor details from those in Galactinia succosa. In this fungus, according to Maire(15,19), the asci arise from a filament of binucleated cells which itself arises from a large multinucleated hypha. The hypha with binucleated cells may also branch and thus produce quite a series of asci.

The nuclei of the binucleated cells show conjugate division as in the rusts, (2,3), cross walls are put in, and thus rows of "synkaryons" are formed. The end cell of each row becomes an ascus in which the two nuclei fuse. In the absence of observations regarding the origin of the entire ascocarp, it is as yet premature to draw conclusions as regards the significance of the fusion in the ascus in this case; however, one cannot avoid a suspicion that possibly antheridial and oogonial nuclei are formed but do not fuse in the oogonium, remaining separate and passing by conjugate division, through the ascogenous cells until they reach the ascus where the fusion occurs. The process in this case would then at least be conspicuously similar to that in the rusts as described by Blackman (2) and Christman (3).

The two nuclei which fuse in the ascus, according to Maire, bring in two spiremes which join longitudinally and

later fuse, the resulting chromatin thread having twice the thickness of either spireme. This is followed by synapsis, in which the chromatin is massed in a ball at one side of the nucleus. Later the chromatin becomes finely divided and becomes aggregated in four masses which he calls protochromosomes. These are transformed into four longitudinally split chromosomes. A center now appears on the interior of the nucleus but against its membrane, at the summit of an intranuclear aster. The center divides, the two halves move in opposite directions, and a spindle is formed between them on which the chromosomes are arranged. Radiations are meanwhile formed in the cytoplasm about the elongating nucleus as a center, which become more prominent at its two ends, and finally form the polar asters of the complete spindle. The asters and spindle are thus of different and independent origin. The halves of the split chromosomes on the equatorial plate separate, four passing to each pole of the spindle. These divide longitudinally again during the metaphase, so that eight distinct rods are found at each pole in the diaster stage. Synapsis, and the double longitudinal division of the chromosomes, according to Maire, characterize this as a heterotypic division.

The second division, which he regards as homoeotypic, begins with a spireme which breaks up into eight protochromosomes; these again form four double chromosomes

which separate in the metaphase, and four chromosomes are found at each of the opposite poles. In the third, or typic division, four chromosomes are formed directly from the spireme, without the intervention of protochromosomes, except in Pustularia vesiculosa, in which Maire finds a transition between the typic and the homoeotypic mitosis. The intranuclear achromatic figure and the extranuclear asters are formed in the same manner for all three divisions. Maire agrees thus with the view many times expressed, that the ascus is a spore mother-cell comparable to the mother cell ~~that~~ of a ~~the~~ moss or fern.

The eight nuclei become beaked, and Maire holds that spore formation is effected in the manner described by Harper.

Guilliermond (7,8) has also investigated Pustularia vesiculosa and Galactinia succosa, studied by Maire, as well as Peziza Catnius, Peziza rutilans and Aleuria cerea, and corroborates many of Maire's observations, differing from him, however, particularly as to the number of chromosomes, and the method of their separation in the metaphases.

He accepts Maire's designation of the three divisions in the ascus as heterotypic, homoeotypic and typic. The first division is always preceded by synapsis and further characterized by a double longitudinal splitting of the

chromosomes, distinguishing features of the heterotypic division. According to Guilliermond, the half-chromosomes resulting from the second longitudinal splitting do not separate completely in the metaphase, as described by Maire, but reach the poles as V's or U's as in the Phanerogams (1).

Guilliermond (8) frequently alludes to the difficulty of determining exactly how the chromosomes divide and the halves separate, and when one considers their minute size it is not surprising that the two investigators disagree as to the details of the processes, or that the figures are not at all convincing. However, Maire's previously stated hypothesis that the Ascomycetes have but four chromosomes (16), and his later attempts to prove the doctrine, may account for his method of the separation of the half-chromosomes in the metaphases, as well as the protochromoses in the prophases of the first and second divisions.

Peziza Catnius and Peziza rutilans have sixteen chromosomes, Galactinia has four, but in Pustularia vesiculosa Guilliermond still holds that eight chromosomes are present, against Maire's view that there are four.

As regards the origin of the center, Guilliermond agrees with Maire (14,19, 8). The first indication of the formation of the karyokinetic figure, is the appearance of a

center just within the nuclear membrane, with abundant fibers extending in toward the chromosomes. Both the figure and the description of this stage suggest that he may have had before him only one-half of a spindle which was really in the equatorial plate stage, and whose other pole should appear in the next section. The center divides and the spindle is formed in the usual way. The polar asters are faint and at times cannot be seen except in the third division, where they are always strongly developed, and later are active in the formation of the spores.

Harper has recently extended his studies on the mildews to Phyllactinia (13) and finds there that the development of the ascogonium from a fertilized egg, the budding out of the ascogenous hyphae, and the origin of the asci are essentially the same as in Erysiphe and Sphaerotheca (11,10).

In Phyllactinia, the nuclei in every stage throughout the life history of the fungus show central bodies, and furthermore the chromatin is always oriented on the center.

The center is described as a disc-shaped body lying on the periphery of the nucleus, or in a slight depression of the nuclear membrane. The chromatin is always attached to the center, but its exact arrangement cannot be made out in the vegetative hyphae and young ascogone as clearly as in the larger nuclei of the ascogenous hyphae and asci, where

the number of strands which radiate from the ce ter into the nuclear cavity can be counted.

In the fusing nuclei of the ascus, the centers fuse into one, and the eight chromatin strands of each nucleus combine in such a way as to form exactly eight strands in the fusion nucleus.

The nuclear fusion is followed immediately by synapsis, in which the chromatin is drawn up in a mass against the central body. The chromatin emerges from the synaptic condition in the form of a spirem with eight distinct strands attached to the central body. Each strand of the spirem forms one of the eight chromosomes, which are still connected with the center by means of the linin threads.

The central body divides and the two daughter centers in migrating apart to form the spindle poles separate the fibers which connect the chromosomes with the center, so that each chromosome is attached to both centers. This continuous connection between the central body ~~and~~ the chromatin strands, and later with the chromosomes themselves, is further used as evidence that the chromosomes are permanent structures of the nucleus.

Here for the first time the fact that there is a permanent connection between the center and chromosomes has been established. The nucleus is thus shown to be a polarized structure throughout the life history of the

fungus, unipolar in the resting condition and becoming bipolar in division.

The process of spore formation in Phyllactinia is the same as in Erysiphe, and, as is so frequently found in the mildews, only two nuclei are enclosed in spores, the remaining six degenerating.

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The mildews offer ~~an~~ especially favorable material for the study of nuclear fusions, nuclear divisions and the very peculiar process of free cell formation which characterizes the ascus, and the following study was undertaken for the purpose of extending our knowledge of the group by an account of the development of the ascus and spore formation in the genus Microsphaera. I have not undertaken to work over the earlier stages in the formation of the perithecium, but have directed my attention mainly to the question of the persistence of the centers during the processes of nuclear fusion and nuclear division, and to the process of spore formation in the ascus. I have, however, observed incidentally certain stages in the development of the perithecium bearing on the account given by Neger of the structure of the mature ascocarp and its ecological adaptations, and shall describe these observations in connection with my account of the structure of the nuclei in the ascogonium and ascogenous hyphae from which are formed the nuclei that ~~which~~ subsequently fuse in the young asci.

As is everywhere commonly observed in this country, Microsphaera Alni (D.C.) grows in great abundance on Syringa vulgaris - the common lilac, - covering the leaves with a white cobwebby mycelium dotted with the dark specklike fruit bodies, and furnishes an abundance of material in all

stages of development for cytological study. The fungus shows a radial growth, so that infected spots may have nearly all stages, from mature perithecia in the center to the youngest fruits on the periphery.

Small squares of leaf covered with the mycelium and perithecia were fixed in Flemming's stronger solution of chromosmi~~um~~c-acetic acid, Flemming's weaker solution, and Flemming's weaker solution diluted one-half with water. Flemming's weak gave the best results in general, although the younger stages fix well in the diluted solution.

The material was collected in September and October 1904 and 1905. Sections were cut 5μ and 10μ thick and stained with Flemming's triple stain.

I shall describe the structure and the development of the ascocarp from the time when the ascogonium is completely enveloped by the perithecial hyphae, leaving the earlier stages for description later.

The young ascogonium appears as a relatively large single cell, somewhat elongated and curved, surrounded by the first hyphal envelop. This stage in which two nuclei are present is of frequent occurrence. The nuclei lie in the long axis of the ascogone and invariably show distinct centers and a single nucleole.

With the triple stain the centers are usually violet or dark red, and are easily distinguished from the dark

blue chromatin or from the blue gray membrane of the nucleus. The nucleole is always a bright red, the nuclear sap is clear and unstained, while the cytoplasm varies from gray to a faint orange color.

The chromatin at this stage often forms a spindle-shaped mass between the central body and the red nucleole. Chromatic strands cannot be made out, but the chromatin appears rather evenly granular, though plainly connected with the central body(

The ascogone grows both in diameter and length, its nuclei divide and later cell division occurs, so that a multicellular organ is formed of four or five cells. During the growth of the ascogone, the envelop becomes more complex, one or two layers of cells ~~are~~ formed about the first. The ascogone, being hemmed in on all sides by its envelop, curves and turns about as it grows, apparently expanding in whatever direction it finds least resistance, so that a much bent structure results. From just what cell or cells the ascogenous hyphae arise, I have not been able to determine. It appears that many ascogenous hyphae bud out at about the same time. These develop into multinucleated branches of the ascogone. The nuclei ~~are~~ however, soon separated by cell walls, except in certain cases in which two nuclei are included in a single cell. These binucleated cells will later become the asci.

Before the young asci are formed, the perithecium begins to show some differentiation in its hyphal layers. There is an outer layer of wide-lumened cells, ~~which~~ already showing some thickening in their walls, on the upper side of the perithecium. Within are two or three layers of thin-walled cells, smaller and more densely filled with protoplasm. The inmost layer of these is especially active; it grows and sends branches in toward the center of the fruit body, crowding against the ascogenous hyphae, intertwining among them and becoming divided to form the so-called "nurse cells". The nurse cells are ~~one~~ or multinucleated, and are thus seen to have been formed from centripetal branches which are at first multinucleated but are later cut up into smaller cells. This ingrowth of the perithecial cells is practically the same as described by Harper for *Erysiphe communis* (11). Certain binucleated cells of the ascogenous hyphae are meanwhile developing into asci. With their growth, the nurse cells are crowded back and flattened between the asci and the perithecial wall. Tangential sections of half-grown perithecia show these thin-walled cells as polygonal plates with two or more nuclei.

The young asci when first recognizable are little larger than the other cells of the ascogenous hyphae. They present very irregular forms, probably due to the crowded condition within the perithecium at this time, but soon round out their

angles, growing at the expense of the surrounding cells which they push back.

The two nuclei in the young asci, although lying very close together, at first show no tendency to fuse. They have well defined centers to which the chromatin is attached, the strands extending back into the nuclear cavity in a typical cone (fig 5 ~~7~~). The nucleole often lies near the nuclear membrane opposite the center. This antipodal relation of center and nucleole is very common throughout the nuclei of Microsphaera.

The ascus grows rapidly, ~~and~~ at first ~~more~~ in the region farthest from the nuclei, so that these come to lie in the smaller end of the cell. The nuclei also increase in size, but not in proportion to the ascus. When the ascus has reached about one-third its mature size, the nuclei come in contact preparatory for fusion. They are sometimes elongated and ~~one~~ may lie one a little above the other, or in any other position. Finally the walls between break down, and fusion occurs. A late stage in the fusion of the nuclei is shown in fig. ~~4~~ 6. The two centers with their respective chromatin systems are still separate. (The one on the left is cut through). The nucleoli have already fused into one large nucleolus. The ascus at this stage is about half-grown. It is well rounded out except where it presses against an adjoining ascus. The fusion nucleus is about the size of the

average primary nucleus.

The most frequent and conspicuous stage found in *Microsphaera* is that of the primary nucleus. It persists from the relatively early fusion in the young ascus, until the ascus has reached its full development. It grows very little after fusion is complete. There is an abundance of chromatin which readily stains a dense blue. This is at first arranged in irregular strands which occasionally appear double, and always cross and interweave in a tangled net. A large nucleolus is always present, most frequently slightly flattened against the nuclear membrane. The center is most difficult of demonstration, partly because the chromatin stains so heavily and is so abundant as to hide the center. Moreover, metachromatic bodies are particularly abundant at this stage, especially in cases of poor fixation. These bodies occur just outside the nucleus, often at a point where two or more chromatin strands touch the nuclear membrane, and may obscure the central body.

At a later stage, the chromatin appears much reduced, and lies massed in a ball against one side of the nuclear membrane. From this apparently synaptic mass thin chromatin strands again extend into the nuclear cavity. Finally there appears a well developed spireme oriented on the central body, (fig. 7). In the uninucleated stage, it is only where the chromatin is pretty well washed out that the center

appears as such. It is then a very dark, disc-shaped body pressed close against the nuclear membrane.

The perithecium has now grown to its full size, and has as many cell layers as when fully ripe. The outer layer of cells begins to show a differentiation into an upper and under region, which is due to a thickening of the walls on the upper surface while the cells on the under side remain thin-walled and contain normal protoplasm and nuclei. The cell lumen is diminished by the thickening of the walls and is almost empty of protoplasm, while even at this stage, the walls contain a brown pigment and are hard and brittle. Neger (21) first pointed out this differentiation, and described the thin flexible walls of the lower cells as caving in when the perithecium is dried out, and bulging out as the cells absorb moisture and become turgid. This alternate drying and swelling of the cells would loosen the perithecium from its mycelium.

A secondary mycelium, such as is found in Phyllactinia (13), springs also from these lower living cells, and intertwines with the original mycelium covering the leaf.

The transition cells in the equatorial region, midway between the upper and under halves of the outer layer of the perithecial wall, give rise to the appendages by the extension of their cell walls. These grow out in a circle about the middle of the perithecium and are directed upward from the leaf surface.

Nuclear Divisions.

The primary nucleus undergoes three successive divisions, giving rise to two, four, and finally eight nuclei, all of which form spores.

I find in an early prophase of the first division two centers about 45° ~~degrees~~ apart, each with a large aster of long, fine rays, and a broad brush of fibers extending into the nucleus (fig. 8). The two centers with their asters probably originate here as elsewhere from the division of the single central body of the resting nucleus, and a single aster developed earlier in the prophase. The two sets of fibers meet below the center of the nucleus, where they cross and interlace; some of the fibers appear to be continuous from one center to the other. At the nuclear membrane, where the broad centers are attached to the intranuclear bundles of fibers, there is a conspicuous non-staining region. Some of the peripheral fibers can be traced to the disc but ~~the bulk~~ fade abruptly just before reaching the central body, leaving an apparent space (fig. 8). The so-called "achromatic" fibers at this stage stain quite as densely as the chromatic parts of the nucleus, so that the chromosomes cannot be clearly distinguished. I have not found the later stages of this division.

The binucleated stage of the ascus, following the first division, is easily distinguished from the binucleated condition before fusion, both by the mature size of the ascus, and by the older appearance of the whole perithecium. The two outer cell layers on the upper side of the perithecium have thick brown walls, and the appendages have nearly reached their final length.

A resting nucleus ~~of~~ this stage has a prominent center to which the chromatin is plainly attached; ~~it~~ is always readily seen as a little cap just outside, and closely pressed against the nuclear membrane. ~~(fig.)~~

One of the most common division figures in my material is the equatorial plate stage of the second division. The spindle usually lies transversely in the ascus, with eight chromosomes arranged on the equatorial plate. The asters are inconspicuous, with fine, delicate rays that fade into the cytoplasm. Between the centers and the spindle poles, light areas are found as in the first division. The four nuclei resulting from this division, ~~are not~~ different ~~from the preceding~~ except that they are somewhat smaller. (fig. ~~45~~ 11).

The third division is ushered in by a division of the center. Most frequently the centers are far apart—50° or 60°—when the asters and spindle fibers are well developed. The asters are particularly striking; their rays are like short, stiff bristles, and densely stained. There is the

same colorless space between the center and the darkly stained spindle fibres, as in the two preceding divisions. This whole stage of spindle formation bears a very close resemblance to the same stage in Erysiphe, (6).

There are eight chromosomes on the equatorial plate and many more in the metaphases. The astral rays grow longer and become much finer, and as the spindle often lies close to the ascus wall, they may be seen bending away from the wall toward the interior of the ascus. A small light zone still appears at each pole of the completed spindle.

The process of spore formation in Microsphaera Alni is entirely like that described by Harper in detail for Erysiphe communis (11), and more recently for Phyllactinia suffulta, (13), and corroborated by various authors (8,19) for many other Ascomycetes.

Begin The eight nuclei formed by the third division, retain their asters; ~~which~~ continue to grow ~~into~~ long, fine threads ~~and~~ become more numerous. From the beginning the asters are turned toward the periphery of the ascus, (fig. 12). With the growth of the asters the nuclei become beaked. The center is situated at the summit of the beak and from it chromatin strands run back into the nuclear cavity. During the process of beak formation, the nucleus with its aster shifts its position, so that it lies a little farther in from the ascus wall, (fig. 8). At this time the rays next the nucleus begin

to curve back about it; more bend over in the same way, until a cone-shaped opening is formed in the midst of the aster. This folding back continues until the majority of the fibers lie in one plane, which forms a hemispherical covering over the beaked nucleus. Some of the fibers bend further, pass below this surface, and are finally enclosed within the spore (figs. 13, 15 ~~9 and 11~~).

As yet there is no differentiation of protoplasm within the ascus; the fibers continue to grow in length, cutting through a homogeneous cytoplasm. That the lateral fusion of the rays begins early, as is shown by the plasmolysis of the upper end of the spore in shrunken material, has been pointed out by Harper (13). This shrinkage of the upper end of the spore often occurs long before the spore plasm is delimited and there is no sign of a hyaline zone or any other differentiation in the cytoplasm, to indicate the position of the future spore membrane. The cytoplasm facing the cleft made by the shrinkage is ragged and without a definite boundary, while the end of the spore is smoothly rounded off and has a continuous outline.

The fusion of the rays progresses slowly toward the interior of the ascus, following the longer fibers which have grown past the nucleus and are now converging toward a point opposite the central body and some distance below the nucleus. These advance fibers mark the path of the plasma

membrane, passing into its composition as the fusion progresses. These stages look like Faull's figures of the hyaline zone, but the fibers always stain blue and do not increase in thickness.

The cleft formed by the shrinkage either of the spore or surrounding epiplasm narrows from the center outward along the plasma membrane which covers the upper part of the spore plasm. When the epiplasm is shrunken, it is thickened at its inner edge simulating a membrane, so that at first glance it suggests two plasma membranes developing from the center outward, such as Faull describes. Closer scrutiny, however, proves the absence of a membrane on the surface of the epiplasm facing the opening. This cleft may, of course, almost surround the spore, or only cap it, according to the stage of development of the plasma membrane of the spore.

The kinoplasmic fibers finally meet at a point below the nucleus, having cut through the cytoplasm so as ~~to~~ exactly enclose an ellipsoidal mass of protoplasm, in the upper end of which lies the nucleus still attached by its center to the new plasma membrane, (figs. 13, 14 ~~9 and 10~~). The beak has been greatly elongated, but is still traversed by chromatin strands connected to the centrosome. Sometimes the nucleus is swung to one side and lies against the plasma membrane of the spore, (fig. 14).

After the fibers have completely fused, so that the spore plasm is actually separated from the epiplasm, the center breaks away from the plasma membrane and the beak of the nucleus is slowly drawn in. There remain traces of the rays either as ridges on the plasma membrane or as enclosed fibers which did not take part in the fusion, but these soon disappear (fig. ~~11~~ 15).

~~In~~ resuming its normal spherical shape, the nucleus moves down into the center of the spore mass and lies there in a resting condition, the chromatin in an irregular reticulum always oriented on the large center (fig. ~~12~~ 16).

Between the spore membrane and the surrounding protoplasm, a space appears in which the spore wall is finally deposited. The epiplasm is often thickened along this space but still has no limiting membrane. The spore wall is at first a faint blue line, but when completed it is a thick dense coat and the epiplasm is no longer drawn back from it (fig. ~~12~~ 16).

With the ripening of the spores the perithecium reaches its maturity. The dark thick walls of the cells on the upper surface have become so hard and brittle that they invariably break in sectioning, while the cells on the under side retain their thin walls and appear in normal living condition. How much this differentiation is due to the drying out of the upper exposed surface of the fruit body,

while the lower cells are protected from too great loss of moisture, could only be estimated by comparison with other mildews; however, Erysiphe and Phyllactinia give no evidence on this point, and Neger's view (21,22) as to the functional difference may be accepted for the present. The appendages grow to great length and branch profusely at their extremities. They contain protoplasm and a large elongated nucleus which lies just below the dichotomously branched end. The walls, though thin and transparent, are very brittle.

General Conclusions.

All the stages in the life history of *Microsphaera* studied thus far show that the central body is a permanent structure of the nucleus, and that it is present not only as a definitely differentiated body, but also as a point of attachment for the chromatin.

The central body is nowhere more easily demonstrated than in the nuclei of the vegetative mycelium; it is conspicuous in the ascogone and ascogenous hyphae, and, with the exception of the primary nucleus where it is sometimes obscured by the abundant chromatin content, is a prominent feature of the nuclei of the ascus, both in the resting condition and in division. Finally, it is present during spore formation and in the resting spores.

The chromatin is in every case plainly connected with the central body, either by direct contact or attached by means of kinoplasmic fibers. In the larger nuclei the central body lies at the apex of a cone of chromatin strands, while in the smaller nuclei although the chromatin is plainly attached to the center, the strands cannot be made out and it appears rather evenly granular.

The center is always an extranuclear body, and my observations differ radically from those of Maire and

Guilliermond on this point. In polar and oblique views it may, to be sure, appear, to be within the nuclear membrane, and I am inclined to suspect that, as some of their figures seem to suggest, the intranuclear centers described by Maire and Guilliermond may be accounted for in this way, or the centers may have been actually drawn into the interior of the nucleus as a result of poor fixation. Their descriptions of spindle formation, by the division and migration of the centers, and the differentiation of spindle fibers, agree with the process observed in Microsphaera, and it is to be noted that when the spindle is complete the centers at the poles are described by them as on or very near the nuclear membrane. However, Maire's description of the formation of the polar aster from cytoplasmic fibers which radiate from the nucleus is entirely different from anything I have found in Microsphaera, where the asters consist of kinoplasmic rays formed about the central body just before division occurs.

The synaptic mass, described by these authors, has no such definite position in the nucleus as in Microsphaera. The presence of the central body, which is in continuous connection with the chromatin, locates a polar region where the chromatin must aggregate when contracted.

Maire's further attempt to bring the divisions of the nuclei of the ascus into harmony with the latest views

regarding reduction division in the higher plants has led to his adoption not only of the fusion of two spiremes, as described by Allen (1) for Lilium, but also the pairing of protochromosomes to form the real chromosomes, comparable to the formation of Strasburger's zygosomes (23).

It is also plain in Microsphaera that the delimitation of the spores is accomplished by the activity of the astral rays which persist from the third nuclear division. As described, the growth and increase in number of the astral rays is accompanied by the formation of a beak on the polar end of the nucleus (fig. 12). At the same time, the nucleus and aster move away from the ascus wall toward the interior of the ascus. The fibers bend down around the nucleus and grow in a curved line toward a point directly below the nucleus where they all finally meet. Lateral fusion of the rays begins at the polar region and progresses outward toward the base of the spore, forming a complete membrane about the ovoid mass of protoplasm which, with the enclosed nucleus, forms the ascospore. The motion of the fibers through the cytoplasm cannot be due to crowding resulting from an outward movement of the nuclei toward the wall as Faull suggests, for at this time in Microsphaera the nucleus and the aster move in from the ascus wall. His other hypothesis that the "centrosome is a dynamic center and the rays an expression of cytoplasmic activity controlled by the

nucleus" causing the rays "to turn toward the bulk of cytoplasm which lies centrad of the centrosome", is based on a confusion of two entirely separate views of the centrosome, first as a dynamic center, and second as a center of metabolic activity. It seems probable, however, that the rays are something more than cytoplasmic particles arranged along lines of force, since, as described above, after they have begun to fuse they can be separated from the cytoplasm by plasmolysis. Faull's further argument that the bending of the rays throws them further apart rather than brings them closer together, so that fusion is impossible, shows that he is here thinking not of adjacent but of opposite rays. The rays which fuse, of course, are those going to the same side of the future spore.

The figures of the beaked nucleus with its aster within the plasma membrane of the spore, which Faull regards as conclusive evidence that the rays take no part in forming the spores, may be explained, as noted, as polar views of spore formation by astral rays. This can be readily seen by comparing Faull's figures (5, figs. 27, 28, 29, 34, 35) with an oblique view of a spore of Phyllactinia during the formation of the plasma membrane as described by Harper (13, pl. 7, fig. 81). Further, ~~also~~ the mildews regularly show the presence of more or less numerous rays which lie inside the plane of fusion and so exist as free separate fibers within

the plasma membrane after the spore is delimitated, but this ~~is~~, of course, is no evidence that the plasma membrane was not formed by the fusion of other rays of the original aster. In some cases the plasma membrane retains a ribbed appearance such as Meves (20) describes in the formation of the "Schwanzmanchette" in the spermatogenesis of the guinea pig. Traces of the fibers may persist even after the nuclear beak is withdrawn (fig. ~~14~~ 15). Faull assumes that the enclosed rays are either the entire original aster or are newly formed, a conclusion which is, of course, unjustified. His figures of this stage (5, figs. 26, 30, 31) agree entirely with the same stages in *Microsphaera* (Figs. 13 [illegible]). That the rays do actually fuse is proved by plasmolysis such as is found in shrunken material where the spore is pulled away from the cytoplasm. A careful study of Faull's paper leads inevitably to the conclusion that the apparent disagreement of his conception of spore formation with that here described is due not so much to a difference in the figures actually observed, as to a failure on his part to carefully analyze the results of his observations.

Finally, it may be noted that the stages studied give no evidence of the existence of a series of "synkaryons" in *Microsphaera*, such as Maire describes for *Galactinia succosa* (~~19~~ 17). In the perthecium the nurse cells may have two or more nuclei while the binucleated cells of the

ascogenous hyphae become the asci.

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NUCLEAR STRUCTURE AND SPORE FORMATION IN *MICROSPHAERA ALNI*

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NUCLEAR STRUCTURE AND SPORE FORMATION IN MICROSPHAERA ALNI.

M. C. SANDS.

(With Plate XLVI.)

INTRODUCTION.

The literature on the development of the ascus and its cytology has been recently and fully reviewed (4, 13), and I shall refer only to such very recent papers as touch upon the points with which I have been specially concerned.

Faull (5) describes for *Hydnobolites*, *Neotiella*, *Sordaria* and some other species, a method of spore formation which he considers essentially different from that described for the Ascomycetes by Harper (9, 11). He finds the central body by no means a permanent feature of the cell. In *Sordaria*, certain of the resting nuclei show centers, but in the other species centers with asters appear only at the time of division, disappearing in resting stages. Faull believes that the spindles are strictly intranuclear in origin, the spindle poles being the centers from which the long astral rays extend. In the last division Faull finds the same persistence and subsequent bending of the astral rays as has often been described for spore formation, but attaches no importance to the rays or their activity as far as spore formation is concerned.

The first indication of spore formation, according to Faull, is the appearance of a specialized layer of cytoplasm beginning just around the center and developing progressively outward and around the nucleus until it encloses the cytoplasm of the future spore. He compares this limiting layer to the hyaline zones found in the cleavage stages of the protospores of *Pilob-*

olus (12). It thins out from the center, stains differently from the cytoplasm, but is never clearly definable —"a hyaline zone, structureless or very finely granular." Two plasma membranes develop simultaneously on the site of the limiting layer, one about the spore-plasm, the other lining the cavity in which the spore lies. This produces no visible change in the limiting layer, although he thinks it probable that the plasma membranes result from a cleavage of the zone.

The nucleus "grows down into the center of the spore," forming a beak. Just what Faull means here by "growth" is very uncertain, especially as there is no mention of an increase in the size of the nucleus. Harper (11) has suggested several possible methods of beak formation, and seems to regard the activity of the astral rays, in bending down and exerting a pressure on the nucleus, as the most plausible. When the exospore is formed, the nucleus resumes its spherical shape by withdrawing its beak and with it the center and aster, and in this behavior Faull sees conclusive evidence that the rays take no part in the formation of the spore membrane.

Faull's figures of the beaked nucleus with its center and aster all within the spore membrane are very much like the polar or part polar views of spore formation which I have seen in *Microsphaera*, where spore delimitation is certainly accomplished by means of the astral rays. I shall discuss the significance of such polar views further on. Faull, however, regards these figures as proof that the spores are not delimited by the fusion of kinoplasmic fibers, and leaves the question without accounting in any way for the persistence and bending of the rays during the process of spore formation.

Maire's latest paper (19) describes the nuclear divisions in a number of asci, the mitoses in all of which vary only in minor details from those in *Galactinia succosa*. In this fungus, Maire (15, 19) finds that the asci arise from a filament of binucleated cells which itself arises from a large multinucleated hypha.

The nuclei of the binucleated cells show conjugate division as in the rusts (2, 3), cross walls are put in, and thus rows of "synkaryons" are formed. The end cell of each

row becomes an ascus in which the two nuclei fuse. In the absence of observations concerning the origin of the entire ascocarp, it is as yet premature to draw conclusions as regards the significance of the fusion in the ascus in this case; however, one cannot avoid a suspicion that possibly antheridial and oogonial nuclei are formed but do not fuse in the oogonium, remaining separate and passing by conjugate division through the ascogenous cells until they reach the ascus where the fusion occurs. The process in this case would then at least be conspicuously similar to that in the rusts as described by Blackman (2) and Christman (3).

The first of the three nuclear divisions in the ascus is designated as heterotypic, being characterized by synapsis and a double longitudinal division of the chromosomes. The second division, in which eight protochromosomes appear which later form four double chromosomes, he regards as homoeotypic, and the last division as typic. Thus Maire agrees with the view many times expressed that the ascus is a spore mother-cell comparable to the spore mother-cell of a moss or fern.

The achromatic part of the division figures has both an intranuclear and an extranuclear origin. The center appears on the interior of the nucleus but against its membrane, at the summit of an intranuclear aster. The center divides, the two halves move in opposite directions, and a spindle is formed between them on which the chromosomes are arranged. Radiations are meanwhile formed in the cytoplasm about the elongating nucleus as a center, which become more prominent at its two ends and finally form the polar asters of the completed spindle. The asters and spindle are thus of different and independent origin. The eight nuclei become beaked, and Maire holds that spore formation is effected in the manner described by Harper.

Guilliermond (7, 8) has also investigated *Pustularia vesiculosa* and *Galactinia succosa*, studied by Maire, as well as *Peziza Catnius*, *P. rutilans* and *Aleuria cerea*, and he corroborates many of Maire's observations, differing from him, however,

particularly as to the number of chromosomes and the method of their separation in the metaphases.

He accepts Maire's designation of the three divisions in the ascus as heterotypic, homoeotypic and typic. The first division is always preceded by synapsis and further characterized by a double longitudinal splitting of the chromosomes, distinguishing features of the heterotypic division. However, he holds that the half-chromosomes resulting from the second longitudinal splitting do not separate completely in the metaphase, as described by Maire, but reach the poles as V's or U's as in the Phanerogams (1).

Guilliermond (8) frequently alludes to the difficulty of determining exactly how the chromosomes divide and the halves separate, and when one considers their minute size it is not surprising that the two investigators disagree as to the details of the processes, or that the figures are not at all convincing. However, Maire's previously stated hypothesis that the Ascomycetes have but four chromosomes (16), and his later attempts to prove the doctrine, may possibly have influenced his account of the separation of the half-chromosomes in the metaphases, as well as his interpretation of the protochromosomes in the prophases of the first and second divisions.

Peziza Catnius and *Peziza rutilans* have sixteen chromosomes, *Galactinia* has four, but in *Pustularia vesiculosa* Guilliermond still holds that eight chromosomes are present, as against Maire's view that there are four.

As regards the origin of the center, Guilliermond agrees with Maire (14, 19, 8). The first indication of the formation of the karyokinetic figure is the appearance of a center just within the nuclear membrane, with abundant fibers extending in toward the chromosomes. Both the figure and the description of this stage suggest the possibility that he had before him only one-half of a spindle which was really in the equatorial plate stage, and whose other pole should appear in the next section. The center divides and the spindle is formed in the usual way. The polar asters are faint and at times cannot be seen, except in the third division, where they are always

strongly developed, and later are active in the formation of the spores.

Harper has recently extended his studies on the mildews to *Phyllactinia* (13) and finds there that the development of the ascogonium from a fertilized egg, the budding out of the ascogenous hyphae, and the origin of the asci are essentially the same as in *Erysiphe* and *Sphaerotheca* (11, 10).

In *Phyllactinia*, the nuclei in every stage throughout the life history of the fungus show central bodies, and furthermore the chromatin is always oriented on the center. The center is described as a disc-shaped body lying on the periphery of the nucleus, or in a slight depression of the nuclear membrane. The chromatin is always attached to the center, but its exact arrangement cannot be made out in the vegetative hyphae and young ascogone as clearly as in the larger nuclei of the ascogenous hyphae and asci, where the number of strands which radiate from the center into the nuclear cavity can be counted.

In the fusing nuclei of the ascus, the centers fuse into one, and the eight chromatin strands of each nucleus combine in such a way as to form exactly eight strands in the fusion nucleus.

The nuclear fusion is followed immediately by synapsis, in which the chromatin is drawn up in a mass against the central body. The chromatin emerges from the synaptic condition in the form of a spirem with eight distinct strands attached to the central body. Each strand of the spirem forms one of the eight chromosomes, which are still connected with the center by means of the linin threads.

The central body divides and the two daughter centers in migrating apart to form the spindle poles separate the fibers which connect the chromosomes with the center, so that each chromosome is seen to be attached to both centers. This continuous connection of the central body with the chromatin strands, and later with the chromosomes themselves, is further used as evidence that the chromosomes are permanent structures of the nucleus.

Here for the first time the fact has been established that there is a permanent connection between the center and chro-

mosomes. The nucleus is thus shown to be a polarized structure throughout the life history of the fungus, unipolar in the resting condition and becoming bipolar in division.

The process of spore formation in *Phyllactinia* is the same as in *Erysiphe*, and, as is so frequently found in the mildews, only two nuclei are enclosed in spores, the remaining six degenerating.

OBSERVATIONS.

The mildews offer especially favorable material for the study of nuclear fusions, nuclear divisions and the very peculiar process of free cell formation which characterizes the ascus, and the following study was undertaken for the purpose of extending our knowledge of the group by an account of the development of the ascus and spore formation in the genus *Microsphaera*. I have not undertaken to work over the earlier stages in the formation of the perithecium, but have directed my attention mainly to the question of the persistence of the centers during the processes of nuclear fusion and nuclear division, and to the process of spore formation in the ascus. I have, however, observed incidentally certain stages in the development of the perithecium bearing on the account given by Neger of the structure of the mature ascocarp and its ecological adaptations, and shall describe these observations in connection with my account of the structure of the nuclei in the ascogonium and ascogenous hyphae from which are formed the nuclei that subsequently fuse in the young asci.

As is everywhere commonly observed in this country, *Microsphaera alni* DC. grows in great abundance on *Syringa vulgaris*—the common lilac—covering the leaves with a white cobwebby mycelium dotted with the dark specklike fruit bodies, and furnishes an abundance of material in all stages of development for cytological study. The fungus shows a radial growth, so that infected spots may have nearly all stages, from mature perithecia in the center to the youngest fruits on the periphery.

Small squares of leaf covered with the mycelium and perithecia were fixed in Flemming's stronger solution of chromosmic-acetic acid, Flemming's weaker solution, and Flem-

ming's weaker solution diluted one-half with water. Flemming's weaker solution gave in general the best results, although the younger stages fix well in the diluted solution.

The material was collected in September and October 1904 and 1905. Sections were cut 5μ and 10μ thick and stained with Flemming's triple stain.

I shall describe tho structure and the development of the ascocarp from the time when the ascogonium is completely enveloped by the perithecial hyphae, leaving the earlier stages for description later.

The young ascogonium appears as a relatively large single cell, somewhat elongated and curved, surrounded by the first hyphal envelop. This stage, in which two nuclei are present, is of frequent occurrence. The nuclei lie in the long axis of the ascogone and invariably show distinct centers and a single nucleole.

With the triple stain the centers are usually violet or dark red, and are easily distinguished from the dark blue chromatin or from the blue gray membrane of the nucleus. The nucleole is always a bright red, the nuclear sap is clear and unstained, while the cytoplasm varies from gray to a faint orange color.

The chromatin at this stage often forms a spindle-shaped mass between the central body and the red nucleole. Chromatic strands cannot be made out, but the chromatin appears rather evenly granular, though plainly connected with the central body (Fig. 4).

The ascogone grows both in diameter and length, its nuclei divide, and later cell division occurs, so that a multicellular organ is formed consisting of four or five cells. During the growth of the ascogone, the envelop becomes more complex, one or two layers of cells being formed about the first layer. The ascogone, being hemmed in on all sides by its envelop, curves and turns about as it grows, apparently expanding in whatever direction it finds least resistance, so that a much bent structure results. From just what cell or cells the ascogenous hyphae arise I have not been able to determine. It appears that many ascogenous hyphae bud out at about the same time.

These develop into multinucleated branches of the ascogone. The nuclei, however, are soon separated by cell walls, except in certain cases in which two nuclei are included in a single cell. These binucleated cells will later become the asci.

Before the young asci are formed, the perithecium begins to show some differentiation in its hyphal layers. There is an outer layer of wide-lumened cells, already showing some thickening in their walls, on the upper side of the perithecium. Within are two or three layers of thin-walled cells, smaller and more densely filled with protoplasm. The inmost layer of these is especially active; it grows and sends branches in toward the center of the fruit body, crowding against the ascogenous hyphae, intertwining among them and becoming divided to form the so-called "nurse cells." The nurse cells are uninucleated or multinucleated, and are thus seen to have been formed from centripetal branches which are at first multinucleated but are later cut up into smaller cells. This ingrowth of the perithecial cells is practically the same as described by Harper for *Erysiphe communis* (11). Certain binucleated cells of the ascogenous hyphae are meanwhile developing into asci. With their growth, the nurse cells are crowded back and flattened between the asci and the perithecial wall. Tangential sections of half-grown perithecia show these thin-walled cells as polygonal plates with two or more nuclei.

The young asci when first recognizable are little larger than the other cells of the ascogenous hyphae. They present very irregular forms, probably due to the crowded condition within the perithecium at this time, but soon round out their angles, growing at the expense of the surrounding cells which they push back. The two nuclei in the young asci, although lying very close together, at first show no tendency to fuse. They have well-defined centers to which the chromatin is attached, the strands extending back into the nuclear cavity in a typical cone (Fig. 5). The nucleole often lies near the nuclear membrane opposite the center. This antipodal relation of center and nucleole is very common throughout the nuclei of *Microsphaera*.

The ascus grows rapidly, at first mainly in the region farthest from the nuclei, so that these come to lie in the smaller end of the cell. The nuclei also increase in size, but not in proportion to the growth of the ascus. When the ascus has reached about one-third its mature size, the nuclei come in contact preparatory to fusion. They are sometimes elongated and may lie one a little above the other, or in any other position. Finally the walls between break down, and fusion occurs. A late stage in the fusion of the nuclei is shown in Figure 7. The two centers with their respective chromatin systems are still separate. (The one on the left is cut through). The nucleoli have already fused into one large nucleolus. The ascus at this stage is about half-grown. It is well rounded out except where it presses against an adjoining ascus. The fusion nucleus is about the size of the average primary nucleus.

The most frequent and conspicuous stage found in *Microsphaera* is that of the primary nucleus. It persists from the time of the relatively early fusion in the young ascus, until the ascus has reached its full development. It grows very little after fusion is complete. There is an abundance of chromatin, which readily stains a dense blue. This is at first arranged in irregular strands which occasionally appear double, and always cross and interweave in a tangled net. A large nucleolus is always present, most frequently slightly flattened against the nuclear membrane. The center is most difficult of demonstration, partly because the chromatin stains so heavily and is so abundant as to hide the center. Moreover, metachromatic bodies are particularly abundant at this stage, especially in cases of poor fixation. These bodies occur just outside the nucleus, often at a point where two or more chromatin strands touch the nuclear membrane, and may obscure the central body. Faull believes that these metachromatic bodies are normal cell products, since they are always present about the primary nucleus but disappear in later stages. However, this disappearance may indicate merely that fixation is more perfect at these later stages.

At a later stage, the chromatin appears much reduced in volume and lies massed in a ball against one side of the nuclear membrane. From this apparently synaptic mass thin chromatin strands again extend into the nuclear cavity. Finally there appears a well developed spirem plainly oriented on the central body (Fig. 6). In the uninucleated stage, it is only where the chromatin is pretty well washed out that the center appears as such. It is then a very dark, disc-shaped body pressed close against the nuclear membrane.

The perithecium has now grown to its full size, and has as many cell layers as when fully ripe. The outer layer of cells begins to show a differentiation into an upper and an under region, which is due to a thickening of the walls on the upper surface while the cells on the under side remain thin-walled and contain normal protoplasm and nuclei. The cell lumen is diminished by the thickening of the walls and is almost empty of protoplasm, while even at this stage the walls contain a brown pigment and are hard and brittle. Neger (21) first pointed out this differentiation, and described the thin flexible walls of the lower cells as caving in when the perithecium is dried out, and bulging out as the cells absorb moisture and become turgid. This alternate drying and swelling of the cells would loosen the perithecium from its mycelium. A secondary mycelium, such as is found in *Phyllactinia* (13), springs also from these lower living cells, and intertwines with the original mycelium covering the leaf.

The transition cells in the equatorial region, midway between the upper and under halves of the outer layer of the perithecial wall, give rise by the extension of their cell walls to the appendages. These grow out in a circle about the middle of the perithecium and are directed upward from the leaf surface.

The primary nucleus of the ascus undergoes three successive divisions, giving rise to two, four, and finally to eight nuclei, all of which form spores.

I find in an early prophase of the first division two centers about 90° apart, each with a large aster of long, fine rays, and a broad brush of fibers extending into the nucleus (Fig. 8).

The two centers with their asters probably originate here as elsewhere from the division of the single central body of the resting nucleus, and of a single aster developed earlier in the prophase. The two sets of fibers meet below the center of the nucleus, where they cross and interlace; some of the fibers appear to be continuous from one center to the other. At the nuclear membrane, where the broad centers are attached to the intranuclear bundles of fibers, there is a conspicuous non-staining region. Some of the peripheral fibers can be traced to the disc, but most of them fade abruptly just before reaching the central body, leaving an apparent space (Fig. 8). The so-called "achromatic" fibers at this stage stain quite as densely as the chromatic parts of the nucleus, so that the chromosomes cannot be clearly distinguished. I have not found the later stages of this division.

The binucleated stage of the ascus following the first division is easily distinguished from the binucleated condition before fusion, both by the mature size of the ascus, and by the older appearance of the whole perithecium. The two outer cell layers on the upper side of the perithecium have thick brown walls, and the appendages have nearly reached their final length.

A resting nucleus at this stage has a prominent center to which the chromatin is plainly attached; the center is always readily seen as a little cap just outside of, and closely pressed against, the nuclear membrane.

One of the most common division figures in my material is the equatorial plate stage of the second division. The spindle usually lies transversely in the ascus, with eight chromosomes arranged on the equatorial plate. The asters are inconspicuous, with fine, delicate rays that fade into the cytoplasm. Between the centers and the spindle poles, light areas are found as in the first division. The four nuclei resulting from this division do not differ from those of the two-nucleated stage, except that they are somewhat smaller (Fig. 11).

The third division is ushered in by a division of the center. Most frequently the centers are far apart—100° or 120°—when the asters and spindle fibers are well developed. The as-

ters are particularly striking; their rays are like short, stiff bristles, and densely stained. There is the same colorless space between the center and the darkly-stained spindle fibers as in the two preceding divisions (Fig. 10). This whole stage of spindle formation bears a very close resemblance to the same stage in *Erysiphe* (6).

There are eight chromosomes on the equatorial plate and many more in the metaphases. The astral rays grow longer and become much finer, and, as the spindle often lies close to the ascus wall, they may be seen bending away from the wall toward the interior of the ascus. A small light zone still appears at each pole of the completed spindle.

The process of spore formation in *Microsphaera alni* is entirely like that described by Harper in detail for *Erysiphe communis* (11), and more recently for *Phyllactinia suffulta* (13), and corroborated by various authors (8, 19) for many other Ascomycetes.

The eight nuclei formed by the third division retain their asters; from these there continue to grow out long, fine threads which become more numerous. From the beginning the asters are turned toward the periphery of the ascus (Fig. 12). With the growth of the asters the nuclei become beaked. The center is situated at the summit of the beak, and from it chromatin strands run back into the nuclear cavity. During the process of beak formation, the nucleus with its aster shifts its position, so that it lies a little farther in from the ascus wall (Fig. 12). At this time the rays next the nucleus begin to curve back about it; more bend over in the same way, until a cone-shaped opening is formed in the midst of the aster. This folding back continues until the majority of the fibers lie in one plane, which forms a hemispherical covering over the beaked nucleus. Some of the fibers bend further, pass below this surface, and are finally enclosed within the spore (Figs. 13, 15).

As yet there is no differentiation of the protoplasm within the ascus; the fibers continue to grow in length, cutting through a homogeneous cytoplasm. That the lateral fusion of the rays begins early, as is shown by the plasmolysis of the

upper end of the spore in shrunken material, has been pointed out by Harper (13). This shrinkage of the upper end of the spore often occurs long before the spore-plasm is delimited and when there is no sign of a hyaline zone or of any other differentiation in the cytoplasm to indicate the position of the future spore membrane. The cytoplasm facing the cleft made by the shrinkage is ragged and without a definite boundary, while the end of the spore is smoothly rounded off and has a continuous outline.

The fusion of the rays progresses slowly toward the interior of the ascus, following the longer fibers which have grown past the nucleus and are now converging toward a point opposite the central body and some distance below the nucleus. These advance fibers mark the path of the plasma membrane, passing into its composition as the fusion progresses. These stages look like Faull's figures of the hyaline zone, but the fibers always stain blue and do not increase in thickness.

The cleft formed by the shrinkage either of the spore or of the surrounding epiplasm narrows from the center outward along the plasma membrane which covers the upper part of the spore-plasm. When the epiplasm is shrunken, it is thickened at its inner edge simulating a membrane, so that at first glance it suggests two plasma membranes developing from the center outward, such as Faull describes. Closer scrutiny, however, proves the absence of a membrane on the surface of the epiplasm facing the opening. This cleft, of course, may almost surround the spore, or it may only cap it, according to the stage of development of the plasma membrane of the spore.

The kinoplasmic fibers finally meet at a point below the nucleus, having cut through the cytoplasm so as exactly to enclose an ellipsoidal mass of protoplasm, in the upper end of which lies the nucleus, still attached by its center to the new plasma membrane (Figs. 13, 14). The beak has been greatly elongated, but is still traversed by chromatin strands connected to the centrosome. Sometimes the nucleus is swung to one side and lies against the plasma membrane of the spore (Fig. 14).

After the fibers have completely fused, so that the spore-

plasm is actually separated from the epiplasm, the center breaks away from the plasma membrane and the beak of the nucleus is slowly drawn in. There remain traces of the fibers which did not take part in the fusion, but these soon disappear (Fig. 15). While resuming its normal spherical shape, the nucleus moves down into the center of the spore mass and lies there in a resting condition, the chromatin in an irregular reticulum always oriented on the large center (Fig. 16).

Between the spore membrane and the surrounding protoplasm, a space appears in which the spore wall is finally deposited. The epiplasm is often thickened along this space, but still has no limiting membrane. The spore wall is at first a faint blue line (Fig. 16), but when completed it is a thick, dense coat, and the epiplasm is no longer drawn back from it.

With the ripening of the spores the perithecium reaches its maturity. The dark, thick walls of the cells on the upper surface have become so hard and brittle that they invariably break in sectioning, while the cells on the under side retain their thin walls and appear in normal living condition. How much this differentiation is due to the drying out of the upper exposed surface of the fruit body, while the lower cells are protected from too great loss of moisture, could only be estimated by comparison with other mildews; however, *Erysiphe* and *Phyllactinia* give no evidence on this point, and Neger's view (21, 22) as to the functional difference may be accepted for the present. The appendages grow to great length and branch profusely at their extremities. They contain protoplasm and a large elongated nucleus which lies just below the dichotomously branched end. The walls, though thin and transparent, are very brittle.

GENERAL CONCLUSIONS.

All the stages in the life history of *Microsphaera* thus far studied show that the central body is a permanent structure of the nucleus, and that it is present not only as a definitely differentiated body, but also as a point of attachment for the chromatin.

The central body is nowhere more easily demonstrated than

in the nuclei of the vegetative mycelium (Figs. 1-3); it is conspicuous in the ascogone (Fig. 4) and ascogenous hyphae, and, with the exception of the primary nucleus where it is sometimes obscured by the abundant chromatin content, it is a prominent feature of the nuclei of the ascus, both in the resting condition and in division. Finally, it is present during spore formation and in the resting spores.

The chromatin is in every case plainly connected with the central body, either by direct contact or attached by means of kinoplasmic fibers. In the larger nuclei the central body lies at the apex of a cone of chromatin strands, while in the smaller nuclei, although the chromatin is plainly attached to the center, the strands cannot be made out, and it appears evenly granular.

The center is always an extranuclear body, and my observations differ radically from those of Maire and Guilliermond on this point. In polar and oblique views it may, to be sure, appear to be within the nuclear membrane, and I am inclined to suspect that, as some of their figures seem to suggest, the intranuclear centers described by Maire and Guilliermond may be accounted for in this way, or the centers may have been actually drawn into the interior of the nucleus as a result of poor fixation. Their descriptions of spindle formation, by the division and migration of the centers and the differentiation of spindle fibers, agree with the process observed in *Microsphaera*, and it is to be noted that when the spindle is complete the centers at the poles are described by them as on or very near the nuclear membrane. However, Maire's description of the formation of the polar aster from cytoplasmic fibers which radiate from the nucleus is entirely different from anything I have found in *Microsphaera*, where the asters consist of kinoplasmic rays formed about the central body just before division occurs.

The synaptic mass, as described by these authors, has no such definite position in the nucleus as in *Microsphaera*. The presence of the central body, which is in continuous connection

with the chromatin, locates a polar region where the chromatin must aggregate when contracted.

Maire's further attempt to bring the divisions of the nuclei of the ascus into harmony with the latest views regarding reduction division in the higher plants has led to his adoption not only of the fusion of two spirems, as described by Allen (1) for *Lilium,* but also the pairing of protochromosomes to form the real chromosomes, comparable to the formation of Strasburger's zygosomes (23).

It is also plain in *Microsphaera* that the delimitation of the spores is accomplished by the activity of the astral rays which persist from the third nuclear division. As described, the growth and increase in number of the astral rays is accompanied by the formation of a beak on the polar end of the nucleus (Fig. 12). At the same time, the nucleus and aster move away from the ascus wall toward the interior of the ascus. The fibers bend down around the nucleus and grow in a curved line toward a point directly below the nucleus, where they finally meet. Lateral fusion of the rays begins at the polar region and progresses outward toward the base of the spore, forming a complete membrane about the ovoid mass of protoplasm, which, with the enclosed nucleus, forms the ascospore. The motion of the fibers through the cytoplasm cannot be due to crowding resulting from an outward movement of the nuclei toward the wall as Faull suggests, for at this time in *Microsphaera* the nucleus and the aster move in from the ascus wall. His other hypothesis that the "centrosome is a dynamic center and the rays an expression of cytoplasmic activity controlled by the nucleus," causing the rays "to turn toward the bulk of cytoplasm which lies centrad of the centrosome," is based on a confusion of two entirely separate views of the centrosome, first as a dynamic center, and second as a center of metabolic activity. It seems probable, however, that the rays are something more than cytoplasmic particles arranged along lines of force, since, as described above, after they have begun to fuse they can be separated from the cytoplasm by plasmolysis. Faull's further argument that the bending of the rays throws them further apart rather than brings them closer to-

gether, so that fusion is impossible, shows that he is here thinking not of adjacent but of opposite rays. The rays which fuse, of course, are those going to the same side of the future spore.

The figures of the beaked nucleus with its aster within the plasma membrane of the spore, which Faull regards as conclusive evidence that the rays take no part in forming the spores, may be explained, as noted, as polar views of spore formation by astral rays. This can be readily seen by comparing Faull's figures (5, Figs. 27, 28, 29, 34, 35) with an oblique view of a spore of *Phyllactinia* during the formation of the plasma membrane as described by Harper (13, Pl. 7, Fig. 81). Further, the mildews regularly show the presence of more or less numerous rays which lie inside the plane of fusion and so exist as free, separate fibers within the plasma membrane after the spore is delimited, but this, of course, is no evidence that the plasma membrane was not formed by the fusion of other rays of the original aster. In some cases the plasma membrane retains a ribbed appearance such as Meves (20) describes in the formation of the *"Schwanzmanchette"* in the spermatogenesis of the guinea pig. Traces of the fibers may persist even after the nuclear beak is withdrawn (Fig. 15). Faull assumes that the enclosed rays are either the entire original aster or are newly formed, a conclusion which is, of course, unjustified. His figures of this stage (5, Figs. 26, 30, 31) agree entirely with the same stages in *Microsphaera* (Figs. 13, 14, 15). That the rays do actually fuse is proved by plasmolysis such as is found in shrunken material where the spore is pulled away from the cytoplasm. A careful study of Faull's paper leads inevitably to the conclusion that the apparent disagreement of his conception of spore formation with that here described is due not so much to a difference in the figures actually observed, as to a failure on his part to analyze carefully the results of his observations.

Finally, it may be noted that the stages studied give no evidence of the existence of a series of "synkaryons" in *Microsphaera,* such as Maire describes for *Galactinia succosa* (14,

19). In the perithecium the nurse cells may have two or more nuclei, while the binucleated cells of the ascogenous hyphae become the asci.

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EXPLANATION OF PLATE XLVI.

Figs. 1, 2. Vegetative cells. Nuclei showing relation of chromatin and centers.

Fig. 3. Hyphal cell from the mycelium, showing nucleus with central body.

Fig. 4. Young ascogone showing one nucleus with its center, and one of the cells of the hyphal envelop.

Fig. 5. Young ascus, with two nuclei before fusion.

Fig. 6. Primary nucleus, spirem stage.

Fig. 7. Late stage in fusion of the two nuclei; centers and chromatin systems still separate. Ascus about one-half mature size.

Fig. 8. Spindle formation, first division of primary nucleus.

Fig. 9. Spindle, second division.

Fig. 10. Formation of spindles in third division.

Fig. 11. Four-nucleated stage, two of the nuclei showing chromatin oriented on the central bodies.

Fig. 12. Eight-nucleated stage, showing the persistent asters and the folding over of the rays.

Fig. 13. Stage in spore formation; two spores completely delimited traces of the rays remain.

Fig. 14. Spore formation, showing extreme lengthening of nuclear beak.

Fig. 15. Nucleus resuming spherical form by withdrawal of beak. Traces of astral rays.

Fig. 16. Complete spores, wall being deposited.

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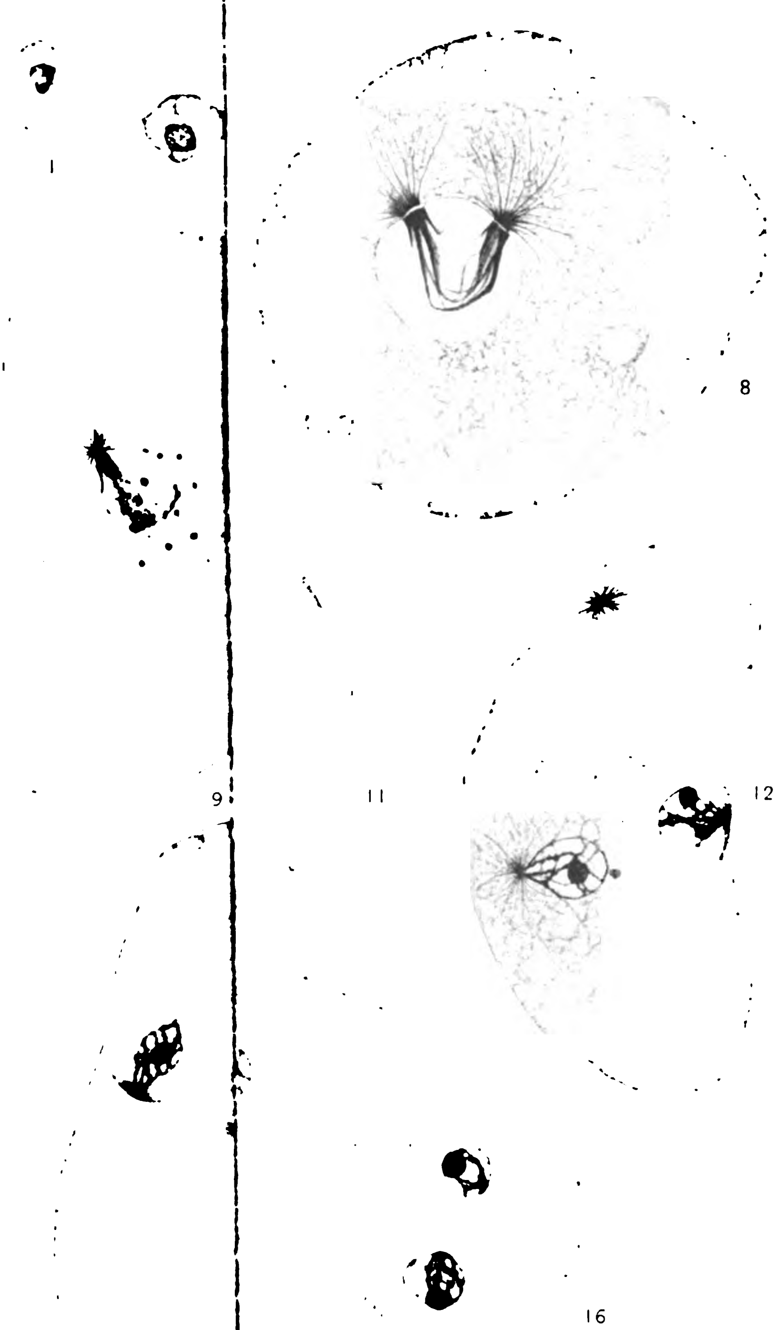
Plate XLVI.



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